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CYTOLOGICAL STUDIES ON THE ENTOMOPHTHOREAE.

I. THE MORPHOLOGY AND DEVELOPMENT OF EMPUSA.[1]

EDGAR W. OLIVE.

(WITH PLATES XIV AND XV)

THE general development of the Entomophthoreae and the external morphology of its various members have been studied by a number of investigators. For a detailed review of the literature pertaining to the group, the reader is referred particularly to the papers of BREFELD ('71,'81,'84) and of THAXTER ('88).

COHN's ('55) results in his classical paper on the developmental history of *Empusa muscae* have been in certain respects considerably modified by later investigation. According to him, the fly first became diseased and the fungus followed as a consequence. The first indications of the disease which COHN could find in the blood of the fly were numberless minute globular or irregularly shaped bodies, whose presence he could not explain otherwise than by the assumption of spontaneous generation (p. 334).

These bodies, according to him, grow larger, become globular or ellipsoidal, and finally grow into the filament, which, by the formation of partitions, becomes the three-celled hypha characteristic of the mature fungus and consisting of spore, stalk-cell, and root-cell. This three-celled character of the hyphae of *Empusa muscae* was disproved, however, the next year by LEBERT ('56) and later by BREFELD and others.

Every investigator who has attempted to infect insects artificially has testified to the difficulties which he has encountered. COHN ('55, p. 342), in speaking of his own lack of success, wisely emphasizes the caution which should characterize such experiments, noting that one should be certain that the insects experimented with are not already stricken with the disease, a more difficult task than would at first appear.

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[1] I am under obligation to the Carnegie Institution of Washington for grants, which have rendered possible this investigation.

BREFELD ('70, '71, '77), however, was successful in transmitting the parasite through external inoculation of spores, and he found that in the case of *Empusa muscae* infection took place only through the thinner whitish parts of the skin on the under side of the fly's body; whereas in another species, *E. sphaerosperma*, the infected hyphae gained an entrance at any part of the skin of the cabbage larva. This author has contributed more on this point than any other, in that he was able to germinate the conidia in artificial media as well as on the surface of the insect body, and to find with the microscope the germinating hyphae actually boring through the skin of the host. In his series of articles, BREFELD has described the complete course of development of two species of Empusa, *E. muscae* Cohn and *E. sphaerosperma* Fres., which furnish quite different types of vegetative growth. According to him, in *E. muscae* there are formed from those germ-tubes which have penetrated into the body-cavity of the insect numerous detached non-nucleate cells, which reproduce by repeated yeast-like sprouting, and which grow within the fat-bodies of the host. At a certain advanced stage of the development of the fungus, the reproduction of the cells by budding ceases, and each grows at one or both ends into a long unbranched tube, which grows through the body-wall and produces at its external end a single conidium. In the other species, *E. sphaerosperma*, BREFELD found that the germ-tube produces, on the other hand, a copiously branching mycelium with many cross-partitions, which finally fills the body-cavity of the host. At the end of the vegetative period, this mycelium sends out hyphae which grow to the surface, branch digitately, and finally produce acrogenously at each ultimate end a single conidium.

Empusa sphaerosperma further differs from *E. muscae* in producing resting spores. COHN ('55, p. 343) had already suggested, since he could not make the conidiospores of *E. muscae* germinate, that perhaps the conidia themselves required a year of rest. But BREFELD ('70, '71), proved conclusively that the spores of this form were short-lived, living only for a few days; hence his first suggestion in regard to the puzzling question as to the wintering of such a species was that this form was probably heteroecious, and that resting spores were produced in some other host. Later, however ('84, p. 68), he seems inclined to believe that the disease is continued over winter on flies in warmer regions, and that it migrates northward with the insects on the return of summer; the fallacy of which theory THAXTER has pointed out.

THAXTER ('88) in his account of the Entomophthoreae of the United States, gives the results of morphological studies based on a considerable number of new as well as old forms. This author disagrees with BREFELD in regard to certain important points. In the first place, he maintains that the vegetative growth in *E. sphaerosperma* is not filamentous in all cases, as is stated by BREFELD, and he appears to be inclined to think that both the filamentous mycelium and the broken-up, budding segments may occur in the same form under different conditions. He asserts that the usual multiplication of the hyphae is not by branching and continuous growth but by the formation of "hyphal bodies," which "consist of short thick fragments, of very varied size and shape, that are continually reproduced by budding or division, until the insect is more or less completely filled with them." But he continues further: "In cases where a direct mycelial growth follows the entrance of the hypha of germination, if indeed such instances occur, this mycelium must fall to pieces into hyphal bodies, before the commencement of growth the direct object of which is reproduction, in a fashion resembling that above described at a similar stage for Conidiobolus" (p. 140). This conception of reproduction by means of "hyphal bodies," however, for reasons that are stated later in this paper, must be abandoned, at least as a generalization.

The segments of the vegetative hyphae, or "hyphal bodies" as THAXTER terms them, under unfavorable conditions may each form a thick-walled resting chlamydospore; or, when the conditions are favorable, they may at once proceed to develop into the fructifying state. In the latter case, according to this author, each hyphal segment sends out one or two (in some species more) hyphae which develop into conidiophores. In the simplest case, a simple conidiophore grows directly to the outer air and produces a single conidium (*Empusa muscae*, e. g.). Or, the conidiophore may become compound and produce a set of conidia. Or, under very favorable conditions, "a single primary hypha may branch indefinitely, each ultimate branch becoming a conidiophore similar to those of the more simple case just mentioned" (p. 142). A singular method of germination of the "hyphal bodies" occurs in *E. aphidis* and *E. virescens*, according to THAXTER's observations. Spherical bodies, evidently regarded as "hyphal bodies" with highly refractive contents, germinate and send out a mass of hyphae in all directions. In this condition they are said to resemble a head of Aspergillus, although the author does not show in either of his two drawings of the phenomenon any trace or remnant of the central cells or "hyphal bodies" from which the radiating hyphae are said to arise.

The conidium is regarded by THAXTER as having a double wall, and thus is to be interpreted more properly as a simple single-spored sporangium.

MATERIALS AND METHODS.

In March 1904, the writer found in horse-dung cultures in the laboratory a small species of fly belonging to the genus *Sciara*, which was infected with an Empusa. This small fly with its attendant disease has been propagated in horse-dung cultures since that time, and many successive generations of the insect, the larval condition as well as the adult, during the year and more of its cultivation, have furnished a wealth of material for an almost complete cytological and developmental study of this species of Empusa. A number of other forms of the Entomophthoreae, most of them in the fructifying stage of their existence, have been used for comparison, but no others have as yet been traced through their entire life history. Enough has been learned, however, to show the existence of a most interesting series of distinctive variations.

My material has been killed with a variety of fixing agents, mostly with varying strengths of Flemming's chromic-acetic-osmic acid mixture. The insect body was generally cut in two or pricked to allow direct contact of the fixing fluid and the fungus hyphae in the body cavity. The material was sectioned usually 3–6 μ thick and stained with Flemming's safranin gentian-violet orange-G, or with Heidenhain's iron haematoxylin.

Six species of Empusa altogether have been thus studied. These species, determined according to the descriptions in THAXTER's account of the group, are as follows: *Empusa muscae* Cohn, on the common house fly; *E. culicis*, A. Braun, on a small green species of Chironomus; *Empusa* sp., on a large fly; *E. aphidis* Hoffman, on various aphides; *E. americana* Thaxter, on a blue-bottle fly; and one other species, which is the principal one studied in this paper, on the small fly, *Sciara* sp. The ovoid conidia of this last form coincide closely with the description of the conidia of *E. montana* Thaxter. They show decided differences, however, from THAXTER'S drawings of this species; and from *E. ovispora* Nowakowski and *E. echinospora* Thaxter, with which the measurements of the conidia also almost coincide, the form is distinguished by the characteristic zygospores of the latter species. While it is possible that, under certain conditions unknown to me, this species on Sciara may produce zygospores, yet the fact remains that after over a year of continuous observation, I have failed to find any resting spores. It is therefore thought advisable to give a new name provisionally to this species, which will hereafter be referred to as *Empusa sciarae.*

Empusa sciarae n. sp.—Vegetative hyphae forming a branching, septate mycelium, which in advanced stages is cut up into few- (generally 3–5-) nucleate cells. Radial hyphae branched; conidiophores 3–5, bearing at each ultimate end a single ovoid uninucleate conidium with a rounded basal papilla, 12–16 × 18–25 μ. Zygospores unknown.

VEGETATIVE STAGE.

My own observations of purely vegetative stages are concerned with two forms only, *Empusa aphidis* and *E. sciarae*, both of which agree with the type described by BREFELD for *E. sphaerosperma.* In all the other species studied, the vegetative condition had ceased and conidiophores had grown out from the vegetative hyphae. As observed by BREFELD, THAXTER, and others, the insect dies at the end of the nutritive period of the fungus; but they do not seem to have emphasized the fact that living insects alone must furnish data for the study of the vegetative hyphae. After the initiation of the reproductive period and the consequent death of the insect, the radial growth of the conidiophores produces a mass of hyphae which might readily be taken, in certain instances, for a vegetative filamentous mycelial growth. The probability has suggested itself to the writer that the fat cells, detached in the search for the fungus, must have been mistaken by some for stages in the development of Empusa; since these bodies may frequently resemble closely short hyphal segments in their fatty, granular contents, as well as in their assumption of globular or irregular shapes, which give the suggestion of budding cells. These detached fat-bodies, which prove in sections to be small aggregates of insect cells, are particularly abundant in the adult just preceding ovipositing. An easy and certain method of distinguishing the vegetative cells of Empusa from fat-bodies which occur with them in the body cavity, is to stain with a dilute methyl-green solution, acidulated with a few drops of acetic acid, when the fungus cells stand out conspicuously, distinguished by their relatively large and characteristic nuclei.

In some preliminary observations on the manner of infection of *Empusa sciarae*, I have not been able to make certain of this point, but I wish to record here some notes of interest pertaining to it. The small fly, *Sciara* sp., was sometimes accompanied, in the vessels in which the dung cultures were kept, by three other species of small flies, which hatched generally in less abundance, *Psychoda* sp., a so-called moth fly, and two other undetermined forms. None, however, other than Sciara were infected, although many times I have noted conidia stuck on the surface of the bodies of the larvae as well as of the adults of the other species. Successive generations of infected Sciara larvae as well as adults have appeared with great regularity every month or six weeks. The fly lays its eggs on the surface of the dung or on the sides of the vessels, and the young larvae, soon after hatching, crawl below the surface of the substratum. It would appear reasonable to assume that in this case infection would occur with ease in this very young condition, when the larval skin is thin and delicate, and before they had crawled below the surface of the dung, where they would obviously not be reached by shooting conidia. My preliminary unsuccessful experiments at infecting healthy adult larvae, confined for a week in bottles with diseased ones, at least suggest the possibility of infection occurring in young insects only. If the cultures in which young larvae were being nourished were kept quite moist, perhaps the majority of individuals in this stage were killed by the disease. When, however, the conditions were drier, the insect developed frequently into the adult fly, which itself, after ovipositing, generally died of the disease.

In order to determine whether the disease could be carried from larval to adult condition, I have examined many pupae, but only in one or two instances have hyphae been found. In one case in which a young insect was struggling to get out of its pupal shell, a few Empusa hyphae were noted when the body was cut open, together with many small eggs and globular fat-bodies. When cultures were kept at some distance from infected ones, in an adjoining room, Sciara frequently developed to maturity by hundreds and died finally a natural death, evidently without infection.

Resting spores in this species, as in *E. muscae*, have not been observed; hence the puzzling question confronts us here also as to the method of wintering of the fungus. An interesting suggestion is made in this connection by the spontaneous appearance of *Empusa sciarae* in laboratory cultures as early in the spring as March, in 1904. This fact apparently still further renders useless BREFELD'S hypothesis as to the migration of the fungus north from warmer countries. The host, in this case, must have been breeding in the dung of the warm stables all through the winter, and it is quite reasonable to suppose that the short-lived fungus must have been continued at the same time on successive generations of insects. The successful cultivation of the form for more than a year in the laboratory gives additional reason for this belief. It is possible that *Empusa muscae* and such similar forms may have lost their sexual stage, because of their success in propagating the disease by means of conidia alone. It is well known that a few house-flies survive the winter by hibernation or otherwise, and it is probable that some of the individuals during the winter may continue breeding in stables or in other favorable places, and in this way carry over the disease, even in cold climates.

Living larvae of Sciara furnish beautiful material for a study of the vegetative conditions of this type of Empusa. When placed on a slide in water, a glance with the naked eye is sufficient to determine whether the larva is infected or not, since the diseased individuals are whitish in appearance, due to the presence of the more or less copious mycelium, while uninfected ones are transparent. Under the microscope, the diseased larvae show clearly the long, branched, mycelial filaments, which in a young condition may have but few septa, while in a later vegetative stage they develop numerous septa. As the larvae crawl about over the surface of the slide, the hyphae can be seen sometimes extending the full length of the body-cavity, sometimes copiously developed in the posterior portion only, again more abundant in the anterior region. As the body parts move, or the blood flows in the cavity, the mycelium shows corresponding pulsations and movements. *Fig. 1* shows a portion of a branched vegetative hypha, taken from such a larva which was crawling just below the surface of the dung culture. Evidently the vegetative activities have here almost ceased, and the 2-, 3-, or 4-nucleate cells are about ready to send out the radial conidiophores. *Fig. 2* shows a section of a younger hypha, in which the cells contain a varying number of nuclei; while *fig. 3* represents a still younger stage, the earliest condition within the body of the host which I have succeeded in obtaining, growing in a larva in which the mycelium was composed of but a few scattered filaments. The section of the hypha, of which *fig. 3* represents only a portion, shows in the preparation twenty-two nuclei, but in the entire length, as far as traceable, not a single cross partition.

While in my own investigations of the vegetative stages of *Empusa sciarae* there remains as yet a small gap, from the penetration of the infecting hypha to the production of such multinucleate mycelial filaments as are shown in *fig. 3*, I feel reasonably certain as to the method of procedure. Germinating conidia, growing in a sterilized decoction of cooked larvae, are shown in *figs. 4–9*. *Figs. 4–7* illustrate the germination of the uninucleate conidia into germ-tubes which have grown out of the liquid decoction, thus resulting in the formation at once of secondary conidia; *fig. 7* shows the beginning of the formation of a tertiary conidium. In *figs. 8* and *9* the germ-tube has grown into a hypha in which all of the protoplasm appears to be in the end cells; in the latter, sixteen apparently empty cells separate the old conidial wall from the terminal protoplasm, which still remains uninucleate.

Both BREFELD ('71, *figs. 5, 29, 31*) and THAXTER ('88, *fig. 240*) give figures showing a somewhat further advance over what I have obtained in the cultivation of conidia, in that in their forms branches are beginning to appear. Whether an increase of the amount of protoplasm and of the number of nuclei has accompanied this branching is doubtful.

It is highly probable, therefore, that in *Empusa sciarae* the hypha which has penetrated into the body-cavity of the fly from the germinating conidium grows rapidly at the expense of the nutrient fluids in which it floats. After the protoplasm has increased in volume, the nuclei increase in number by division, and from the uninucleate condition, in the case of *Empusa sciarae*, the hypha finally becomes a multinucleate branching filament, such as is shown in *fig. 3*. Partition-walls in this form at first occur but sparingly; later, however, at the culmination of vegetative activity, septa are abundant and branching becomes more frequent. Finally the body-cavity of the insect becomes completely filled with the mycelial filaments, vegetative activity ceases, and the fructifying state begins.

Empusa aphidis furnishes a somewhat modified vegetative development. The advanced condition, which is my only source of information in this case, shows in sections branched coenocytic hyphae, which appear to be but rarely divided by septa, unlike the corresponding stage in *Empusa sciarae.* Even after the rhizoids have grown out from the under side of the body of the insect (*figs. 10, 11*), the vegetative activities appear to continue, as evidenced by the fact that the nuclei in this instance are still undergoing division. Also in *Empusa* sp. there occurs a similar prolongation of vegetative activity, since at an advanced stage even fewer cross-partitions can be found in the coenocytic mycelium (*figs. 23, 25*).

REPRODUCTIVE STAGE.

At the culmination of vegetative growth, the body-cavity of the larva appears to be completely filled with a mass of long hyphae. Toward the close of this stage, the larva crawls to the surface of the substratum or high up on the side of the culture dish; or, in the case of the adult Sciara, the fly seeks a conspicuous position, as is common with such infected insects, and death ensues with the beginning of the fructifying condition.

The initiation of the fructifying condition is marked in *Empusa sciarae* by the sudden formation of radial branches from the short cells which make up the hyphal filaments. These branches appear to be put forth more or less simultaneously, in this species but one from each cell. The cell swells up and becomes rounded off somewhat at its ends (*fig. 13*). One or more vacuoles appear in the protoplasm and a protuberance is pushed out from one end of the cell; this grows into the radial hypha destined to become the branched conidiophore (*figs. 12, 14, 15*). The two forces, the swelling and consequent rounding off of the cells at the ends and the pushing out of the branch, combine to split the partition-walls between the cells, thus causing the hyphae to become easily broken up into one-celled segments, the "hyphal bodies" of THAXTER (*figs. 1, 12*). I have seen similar hyphal segments, forming in these instances also the origin of the radial conidiophores, as well in my preparations of *E. culicis* and *E. americana;* but other species promise interesting variations from this common type of pre-reproductive development. In *Empusa* sp. and *E. aphidis*, for example, the vegetative hyphae remain, up to the very initiation of the reproductive stage, either unicellular, or at least with cross-partitions at only rare intervals. In such instances, therefore, no breaking up into "hyphal bodies" occurs; but the vegetative hyphae appear to grow out directly into the conidiophores.

As BREFELD and THAXTER have pointed out, the first hyphae to appear in the external growth of the fungus form the rhizoids, by means of which the host is fastened firmly to the substratum. In the common house-fly, the host is attached by means of its proboscis. In *E. sphaerosperma*, according to BREFELD, bundles of rhizoids break out more or less irregularly from the under side of the body of the insect and attach it to the substratum. According to THAXTER, these rhizoidal hyphae may branch, and may terminate in a kind of expanded sucker, which apparently secretes a viscous substance.

In *Empusa sciarae*, rhizoids are developed more or less abundantly from the under side of the abdomen of the fly, or, in the case of the larva, from almost any point on the under side of the body. In certain forms of *E. aphidis*, groups of rhizoids break out from the under surface of the insect and form large sucker-like hold-fasts. In several instances, I have counted three of these hold-fasts from aphides parasitic on Solidago. *Fig. 10* shows one of these sucker-like bundles of rhizoids in section; and *fig. 11* a highly magnified hypha from near the inner region of the hold-fast. The latter presents clearly the character of the typical rhizoid; thick, frequently yellowish walls, containing but a thin layer of protoplasm, which bounds externally the large vacuolar cavity that almost fills the hypha. The walls of the ordinary vegetative hyphae, on the other hand, are thin; those of the rhizoids in this case appear to have undergone a gelatinous or slimy modification, and the contents seems to be undergoing degeneration.

The growth of the conidiophores, in the case of *E. sciarae*, proceeds more slowly than that of the rhizoidal hyphae, a phenomenon which is probably due, in part at least, to the slower absorption of water by those hyphae destined to bear conidia. At any rate, the vacuoles which are formed at this time in the cells increase slowly in size, and a conidiophore arises from near the end of each cell and grows out radially, boring its way through the tissues of the host (*figs. 12, 14, 15*). As THAXTER and others have noted, the conidiophores of certain species remain simple and unbranched, as in the case of *E. muscae* (*fig. 40*); or, in other species, they may become normally septate and branched, as shown in *E. sciarae* (*fig. 16*), and *Empusa* sp. (*fig. 23*). From this fact arise most interesting cytological variations in the various conidia of these species. THAXTER has brought out clearly the variation in size, shape, etc., of the conidia of many species, but CAVARA ('99) was the first to contrast the multinucleate condition of the conidia of *Empusa muscae* with that of the uninucleate conidia of *Entomophthora Delpiniana*. Of the six species studied by me, four have uninucleate conidia (*E. sciarae, fig. 27; E. americana, figs. 36, 37; E. aphidis, figs. 42, 43;* and *Empusa* sp., *figs. 22, 26*). The conidia of *E. culicis* are normally two-, rarely three-nucleate (*figs. 31, 32*); while those of *E. muscae* have a more or less indefinite number, frequently about 15–18 (*figs. 38, 39*).[2] The more common uninucleate conidia arise primarily from the septation of the conidiophores into uninucleate segments; whereas, on the other hand, the simple conidiophore of *E. muscae* does not usually become septate except at the conidium. In the last case, therefore, all of the many nuclei of the last-formed vegetative cell, which forms the origin of the conidiophore, flow out into the single, large, bell-shaped conidium (*fig. 39*).

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[2] While convinced of the value of CAVARA's suggestion as to the use of nuclear characters in the classification of the Entomophthoreae I do not think that this one character alone would justify the separation of Entomophthora from Empusa.

In *E. culicis*, which has binucleate conidia, septation of the conidiophore occurs, by which the protoplasm cuts itself off from behind; but, unlike the case of *E. sciarae*, I have found no branching with it, so that as a consequence, all of the binucleate protoplasm of the conidiophoric hypha flows into the one terminal conidium (*figs. 30–35*). The simple conidiophore of *E. culicis* resembles, therefore, that of *E. muscae* in being unbranched, but the origin of the conidiophores may differ in the two cases. One may find, in fact, in *E. culicis*, two sorts of "hyphal bodies;" either small cells which, like the corresponding ones of *E. muscae*, give rise to but one conidiophore; or, on the other hand, larger cells, which may give rise to several conidiophores by budding from several points simultaneously, in a similar manner to Conidiobolus. In both instances, each conidiophore remains simple and ultimately bears, terminally, the binucleate conidiospore.

In the species with uninucleate conidia, *E. sciarae*, *E. americana*, *E. aphidis*, and *Empusa* sp., the coenocytic conidiophore, as was indicated above, is cut up by septa, in a manner to be described later, into uninucleate segments (*figs. 16–21*). In all these cases, this results in a branching growth and successive abjunction of the acrogenous spores. Below the terminal cell the penultimate cell pushes out to one side, and thence bores its way to the surface of the host, where it abjoints a single uninucleate conidium (*figs. 16, 23*). From this habit in certain forms, arises a profuse system of branching, frequently of a digitate type (e. g., *Empusa* sp.) or corymbose (*E. sciarae* and others), to enable the uninucleate segments to reach the surface and to discharge their protoplasm by means of the abjointed conidia. But the last-formed vegetative cells of *E. sciarae* contain only 2–4 nuclei (*figs. 1, 12, 15*), so that, in this instance, only a correspondingly small number of branches are formed.

The process of abjection of the conidia of Empusa is apparently similar in a general way to that described for the sporangia of Pilobolus, except that in these conidia there is no gelatinous collar visible. The formation of the partition at the base of the conidium in Empusa also is quite a different process from the formation of the columella in the case of Pilobolus.

In Empusa, the vacuole which appears in the basal portion of the cell becomes larger and larger (*fig. 27*), and a small protuberance, which has a diameter equal to about half that of the conidiophore, is pushed out from the end (*figs. 34, 41*). There is now formed at the apex of this narrowed sterigma a swelling (*figs. 27, 35, 39, 42*), which, after continued enlargement, finally receives the greater part of the protoplasm and all of the nuclear content from the basal portion. The process of cell-division, by means of which this apical conidium is cut off by a wall from the penultimate cell, will be described in some detail later, in connection with the description of cell-division in Empusa. Certain points should be noted here, however, and among them, that the term *basidium*, as applied to the penultimate cell, although in common use in this connection, should, in my opinion, be confined to the Basidiomycetes, where, morphologically, the true basidium is a very different spore-bearing structure from the penultimate cell bearing the conidium in Empusa.

The penultimate cell forms the explosive mechanism by which the conidium is shot off. As the ring-formed wall which cuts through the base of the conidium travels progressively inward, the protoplasm passes through the narrowing opening leading from below, until at the close of abjunction, the basal cell retains only a thin parietal layer of protoplasm, but no nucleus. Continued swelling, due to the absorption of water, finally results in the bursting of the basal vesicle, thereby breaking the wall of the vesicle where it joins that of the conidium. In some forms, a ring-shaped scar is noticeable near the base of the conidium, marking the circle where the summit of the swollen basal vesicle was formerly attached. The septum which separates the conidium from the subterminal cell is at first usually pushed upward, thus resembling a columella (*figs. 28, 30, 31, 36*), but when the spore is shot off, this partition-wall reverses its former position, and in the conidium it appears as a prominent papilla (*figs. 29, 37, 43*). When the basal vesicle bursts, its contents are thrown out of the open ruptured end and frequently persists as a slimy covering about the spore, serving in this case, perhaps, the double purpose of protection against excessive evaporation and of sticking the spore to the substratum which it strikes. I have noted that the explosion, in the case of *Empusa sciarae*, sometimes throws the spore a distance of 6^{mm}; while BREFELD has recorded an even greater distance in the case of *E. muscae*, in which the spores are said to be sent as far as $2–3^{cm}$.

I have not yet completely solved satisfactorily to myself the peculiar method of abjection of the spores of my undetermined species of Empusa. I am inclined to think, however, that this method, in certain respects, is unlike that described above. In this form the conidiophores come to the surface, become cut up by septa into uninucleate segments, and proceed to branch profusely (*figs. 23, 25*). The cell terminating each branch pushes out in a peculiar manner. Instead of forming a large basal vesicle as an explosive mechanism in the usual manner, as described above, here the protoplasm appears to cut itself off from behind by means of one or more successively formed walls (*figs. 22, 24, 26*). A minimum of protoplasm seems to be lost in the process, and this cut-off protoplasm soon assumes a peculiar granular appearance. In this condition it is probably dead, for these cut-off cells appear soon to lose their turgescence. It is difficult to conceive of a forcible discharge of the spore in this instance, especially if it be true that the protoplasm of the basal cells is dead and thus incapable, through loss of turgidity, of functioning as an explosive mechanism. The process here rather seems to be that, by means of these successive abjunctions, the uninucleate spores are pushed off with but little force, and that they are probably followed out of the thick, gelatinous wall of the mother hypha by other cells pushed up from below. It may be, however, that further studies on fresh material of this species will change this impression of subterminal proliferation.

Figs. 45 and *46* represent the terminal portion of large hyphae of *E. culicis* which are destined to form resting spores; and *figs. 47* and *48*, two fully formed, thick-walled resting spores. Such hyphae as are shown in the first two figures are distinguished from conidiophores by being much larger, and, further, they contain four or five nuclei, instead of two. I have traced these hyphae in sections back to large "hyphal bodies," but I was unable to follow their complete history. Whether the thick-walled resting spores of this species are therefore true zygospores, or azygospores, as they are termed in THAXTER'S monograph, I am not prepared to say. I have not been able, however, to confirm VUILLEMIN'S assertions (:**00**) as to the nuclear fusions in the azygospores of *Entomophthora gloeospora*.

UNIVERSITY OF WISCONSIN,
Madison.

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EXPLANATION OF PLATES XIV AND XV.

The drawings were made with the aid of an Abbé camera lucida, and for the most part with various compensating oculars combined with Zeiss 2^{mm} apochromatic obj. N. A. 1.30.

PLATE XIV.

FIGS. 1–9, *Empusa sciarae.*

FIG. 1. A freshly killed filament, fixed and stained with acetic methyl green. ×275.

FIG. 2. Section of a younger filament.

FIG. 3. Section of a still younger vegetative hypha. ×275.

FIG. 4. A germinating conidium, cultivated in a decoction of cooked larvæ, forming a secondary conidium. Killed in acetic methyl green. ×575.

FIGS. 5, 6. Conidia with still younger germ-tubes. Killed with acetic methyl green. ×575.

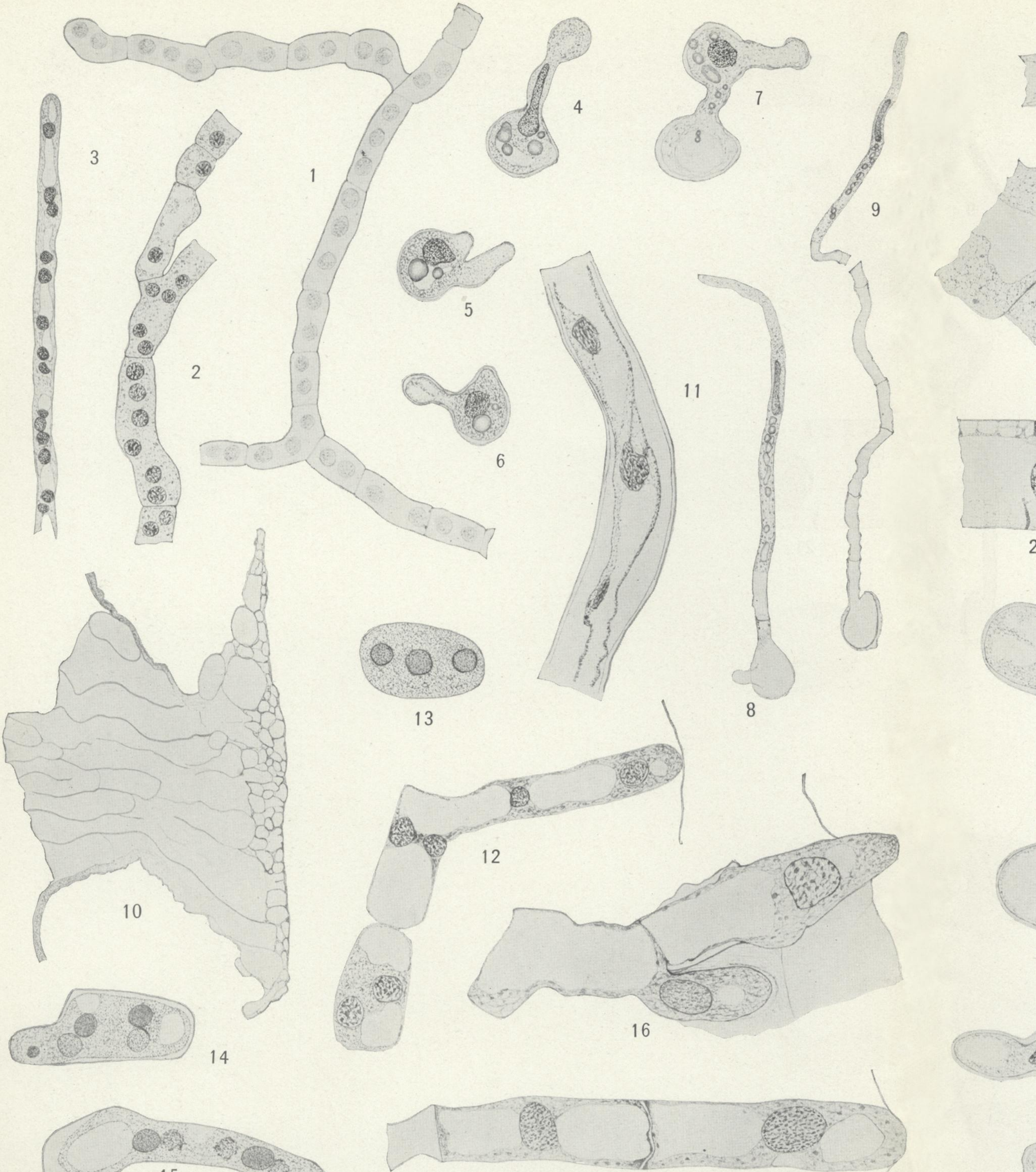
FIG. 7. A germ-tube has formed a secondary conidium, which in turn has started to form a tertiary conidium. Killed ditto. ×575.

FIGS. 8, 9. The conidia have produced long germ-tubes instead of secondary conidia. From the same culture as those above. Killed ditto.

FIGS. 10–11, *Empusa aphidis.*

FIG. 10. A cross-section of one of the sucker-like rhizoidal disks.

FIG. 11. A section of a single rhizoid. ×1080.



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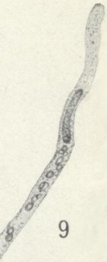
OLIVE on EMPUSA

PLATE XIV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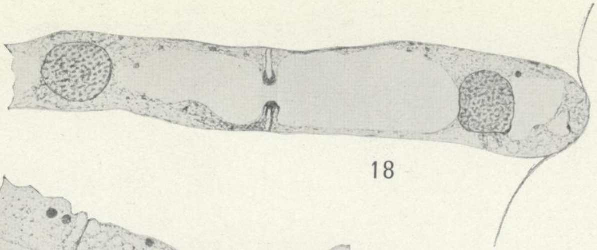
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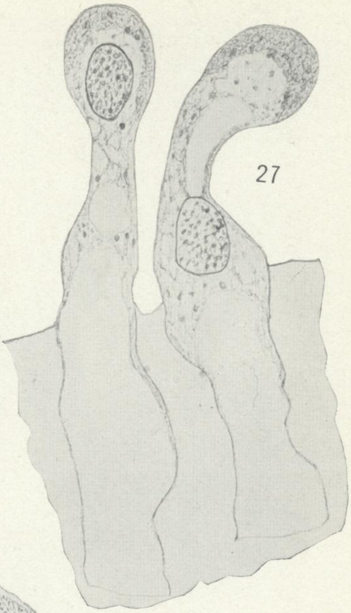
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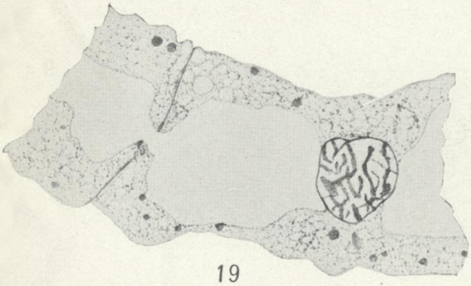
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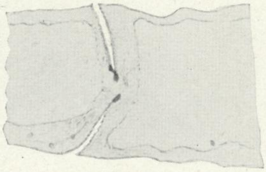
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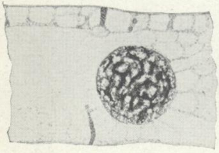
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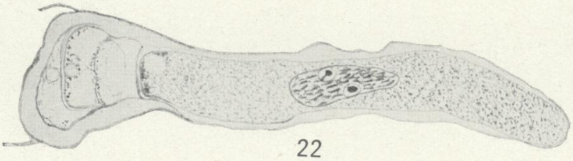
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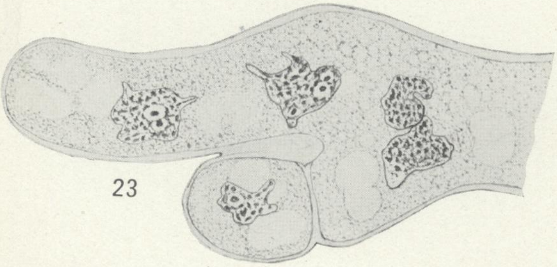
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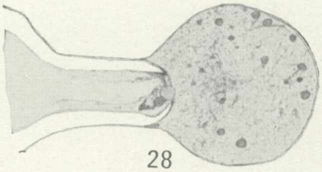
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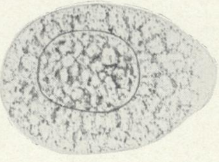
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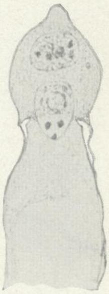
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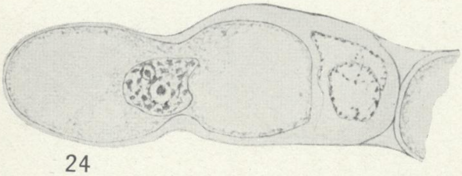
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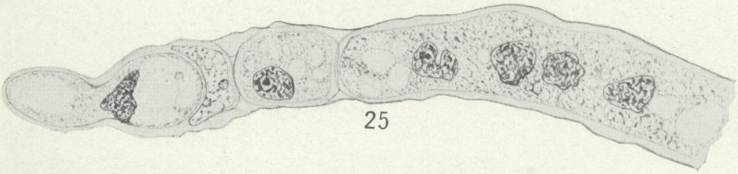
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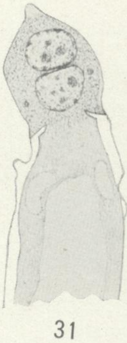
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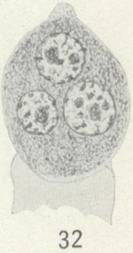
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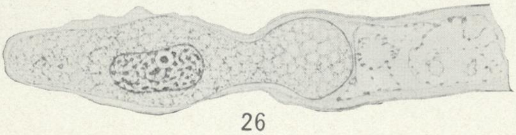
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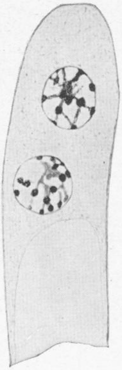
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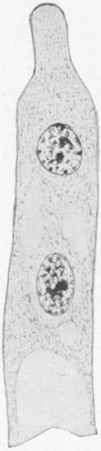
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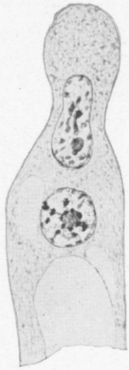
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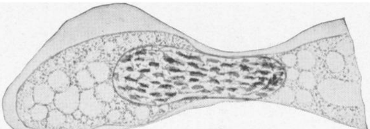
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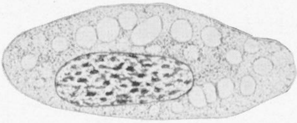
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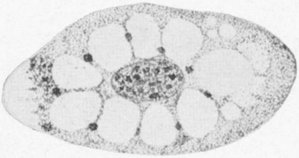
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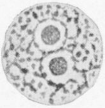
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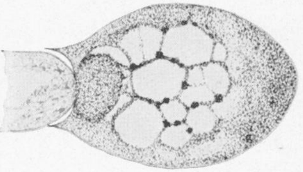
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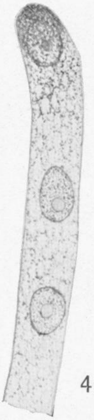
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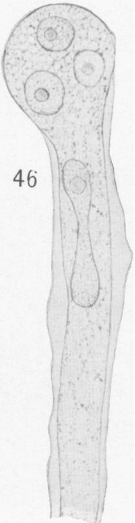
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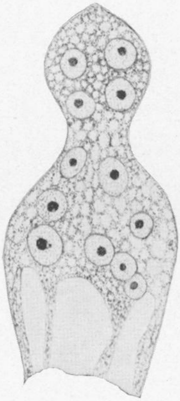
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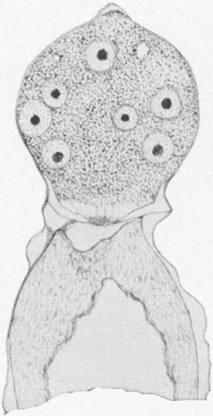
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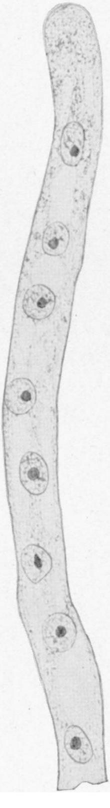
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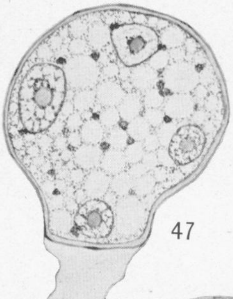
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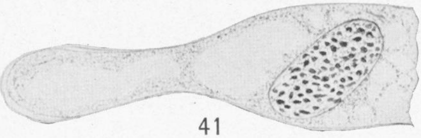
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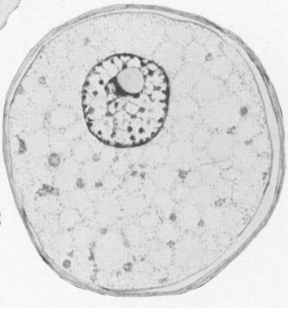
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FIGS. 12–21, *Empusa sciarae.*

FIG. 12. A section showing two cells of the old vegetative mycelium, or "hyphal bodies," from the upper one of which has grown out radially a conidiophore.

FIG. 13. A hyphal segment freshly killed with acetic methyl green. ×480.

FIG. 14. The same, showing the beginning of a conidiophore. ×480.

FIG. 15. The same, showing a still longer conidiophore. ×480.

FIG. 16. Section of a radial conidiophore which is just emerging from the epidermis of the host, showing the subterminal branching. ×1080.

FIG. 17. Section of a conidiophore showing a completed septum between the two cells. ×1080.

FIG. 18. A half-completed stage of cell-division in a conidiophore. ×1080.

FIG. 19. Cell-division in the vegetative mycelium. ×1500.

FIG. 20. An almost-completed stage of cell-division in a conidiophore; the iron-haematoxylin stain has been almost completely washed out of the preparation, except at the innermost margin of the cleft. ×1500.

FIG. 21. A poorly differentiated construction in a vegetative hypha.

FIGS. 22–24, *Empusa* sp. ?

FIG. 22. Section of a conidiophore, showing the peculiar method of the cutting off of basal cells, which apparently soon die. ×1080.

FIG. 23. Section of a conidiophore showing a subterminal branch, and also nuclei which appear to have assumed amoeboid shapes. ×1080.

FIG. 24. A similar section to that shown in fig. 22, but with only one basal cell cut off. ×1080.

FIGS. 25, 26, *Empusa* sp.

FIG. 25. End of a conidiophore, from which has been cut off two uninucleate segments. ×1080.

FIG. 26. A conidium which is apparently being pushed out of the thick, slimy wall of the mother hypha. ×575.

FIGS. 27–29, *Empusa sciarae.*

FIG. 27. A section showing two protruded terminal cells of the conidiophore, from which young conidia are in process of formation. ×1080.

FIG. 28. A section of a conidium in process of abstriction, in which the iron-haematoxylin stain has not differentiated the nucleus but has brought out some of the metachromatic bodies. ×1500.

FIG. 29. A mature conidium.

FIGS. 30–32, *Empusa culicis.* ×1500.

FIG. 30. The upper portion of the terminal cell of a conidiophore, showing at either side the cleft marking the ring-formed cleavage-furrow.

FIG. 31. A section showing a somewhat older stage, in which the cleft has almost abstricted the binucleate conidium.

FIG. 32. A rare occurrence, showing a 3-nucleate conidium.

PLATE XV.

FIGS. 33–35. *Empusa culicis.* ×1500.

FIG. 33. A thin, lightly stained section of the end of a conidiophore, showing the usual binucleate condition.

FIG. 34. An older stage, in which the sterigma is pushing out.

FIG. 35. From a still older preparation, in which the end of the sterigma has become swollen to form the binucleate conidium.

FIGS. 36, 37, *Empusa americana.* ×1500.

FIG. 36. A preparation in which the nucleus is poorly differentiated, but which shows especially clearly the vacuolar cytoplasm and the columella-like wall which finally abstricts the conidium.

FIG. 37. A mature conidiospore, showing the vacuolated cytoplasm (probably filled when living with oil-globules), the single nucleus, and the basal papilla which has been formed by the reversal of the columella.

FIGS. 38–40, *Empusa muscae.* ×1080.

FIG. 38. The partition-wall which cuts off the multinucleate conidium is here completed. The slimy enucleate protoplasm of the basal cell appears to be but little shrunken.

FIG. 39. The end of a young conidiophore, just extruded from the body of the fly, in which the conidium is in process of formation, showing the multinucleate character of the protoplasm.

FIG. 40. A portion of a conidiophore still within the body of the fly, which is growing toward an opening in one of the abdominal joints.

FIGS. 41–44, *Empusa aphidis.* ×1500.

FIG. 41. A section showing at the tip of the conidiophore a young conidium in process of formation.

FIG. 42. An older stage of conidium-formation.

FIG. 43. A mature conidium, showing the uninucleate character.

FIG. 44. A nucleus from a conidiophore, showing two nucleoles, and portions of the chromatic thread.

FIGS. 45–48, *Empusa culicis.*

FIG.. 45. The end of a young azygosporic hypha. ×1080.

FIG. 46. An older condition, which shows the beginning of the formation of a resting spore. ×1080.

FIG. 47. A mature "azygospore." ×1080.

FIG. 48. A thinner section of a mature "azygospore," showing but one nucleus. Other nuclei lie below and above in the section. ×1500.